Efficiency of Hurdle Technology Applied to Raw Cured Meat (Si-Raw) Processing

Ming-Tsao Chen*, Young-Sun Lin[1], Hung-Tsung Tsai[2] and Hsiu-Lan Kuo[3]
Department of Food Engineering, Da-Yeh University
112, Shan-Jiau Rd. Da-Tsuen Hsang, Changhua Sheng, Taiwan 51505, ROC

ABSTRACT : Si-Raw is a raw cured meat (raw, cured meat fermented with steamed rice) produced by the aboriginal people of Taiwan. In order to prevent food poisoning or intoxication from botulism, new methods of monitoring the production base on hurdle technology were investigated. New methods investigated incorporated citric acid, sodium hypophosphite, *Monascus anka* mash, plum paste or lactic acid bacteria inoculum added separately to meat with steamed rice and salt to lower the Aw (water activity) and pH values of the products to control the microbial growth. Results showed that anaerobic bacterial counts, lactic acid bacterial counts and aerobic bacterial counts for the products of all treatments were less than 10^6, 10^5 and 10^2 cfu/g, respectively. Sodium chloride content of all products was above 5.46%, water activity was below 0.939 and pH value was below 4.27. IMP was lower and ATP and hypoxanthine were higher. ATP concentrations were higher in the samples which contained the anka mash. Result of sensory panel test indicated that most people preferred the products with added sodium hypophosphite. Except for the fact that the content of tryptamine in the sample with *Monascus anka* mash was higher, the amine concentrations for all treatments were lower than those of other fermented meat products. The amino acid nitrogen content was higher in the product made from raw meat treated with citric acid, but lower in the other products. Neither *Clostridium botulinum* nor *Trichinella spiralis* were detected in any of the treatments. The result may indicate that hurdle technology is effective for hygiene and safe producing Si-Raw. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 11 : 1646-1652)***

Key Words : Hurdle Technology, Raw Cured Meat, Sensory, Nucleotides, Botulinum

INTRODUCTION

Si-Raw is the raw cured meat product (raw cured fermented with steamed rice) produced in Taiwan aborigines (Umabuchi, 1918). They prefer this type of traditional food. The major effect of this method is to reduce water activity, which inhibits microbial growth and consequently has a preservative effect. During processing the cured meat is placed on a bed of steamed rice in a container to create an anaerobic environment and encourage fermentation to take place, and flavor develops for approximately one month. Ten percent salt based on meat block was used in this experiment according to pilot study. In previous report, some people who consumed this type of raw cured meat were poisoned and three of them died of botulism (Shin and Chao, 1986). In order to prevent food poisoning from botulism, new methods based on hurdle technology (Leistner and Rodel, 1976) were used to produce this kind of product and compared with the traditional method. Si-Raw is made without adding nitrate or nitrite. Therefore, for sanitation concerns, hurdle technology was used to reduce or eliminate pathological contamination of the product by lowering the pH value, Aw and osmolarity during processing. In this study, sodium hypophosphite, a growth inhibitor for *Cl. botulinum* (Wood et al., 1986), and Monascus (Leistner, et al., 1991; Lin, 1986) were added to inhibit bacterial growth, while acid-spraying and inoculation of *L. plantarum* (Okereke and Montville, 1991) were for lowering pH value or bacterial effect, respectively. Alternatively, plum paste was included in the ingredients to decrease the pH value and to provide flavor. Sodium chloride, non-protein nitrogen, amino acid nitrogen, amines, ATP and its related compounds, aerobic bacterial counts, anaerobic bacterial counts, lactic acid bacterial counts and sensory panel scores were determined.

* Corresponding Author: Ming-Tsao Chen. Tel: +886-48528469, Fax: +886-48528466, E-mail: 37002@ms21.url.com.tw
[1] Department of Animal Science, National Chung-Hsing University, 250, Kao-Kaung Road, Taichung, Taiwan 40227, ROC.
[2] Bureau of Food Sanitation, Department of Health, 100 Ai-Kao East Road, Taipei, Taiwan, ROC.
[3] Department of Food Nutrition, Chung-Hwa College of Medical Technology, 89 Wen-Hwa 1 Alley, Jente Hsang, Tainan Sheng, Taiwan 71705, ROC.
Received November 7, 2001; Accepted July 8, 2002

MATERIALS AND METHODS

Pork (ham) obtained from a local Taiwanese meat market was cut into strips approximately $3\times5\times0.5$-$1.5cm^3$ for curing.

Si-raw preparation

Traditional method : Raw pork strips were cured with 10% common salt for 2 days at 3±1°C and the blood and pickle solution was discarded. The precured pork was sprinkled with cooked rice by ratio of 7:3 for total weight of

1 kg. Then. the pork was placed in a glass jar and sealed for fermentation at room temperature (25°C) for 30 days. Cooked rice was removed from the final products before various assays were performed.

Improved methods : Citric acid addition: Raw pork strips were precured with 10% NaCl and 3% citric acid which adjusted pH to below 5.0. The rest of the steps were the same as the traditional method.

Sodium hypophosphite plus citric acid: All procedures were the same as the traditional method. except the step of placing the precured pork on the steamed rice. The rice and precured pork were mixed with 0.3% Na-hypophosphite and the pH adjusted to below 5.0 with citric acid. then the jars were filled and sealed.

Monascus anka mash addition: Precured pork was mixed with anka mash (Monascus *anka* inoculated rice replaced the steamed rice). and this mixture was placed in a jar layer by layer and sealed.

Plum paste addition: The precured pork was mixed with steamed rice and plum paste in the ratio of 14 pork:2 rice: 3 plum paste and filled in a jar and sealed.

Organic acid treatment: Raw meat surface was sprayed with organic acid (a mixture of 0.1% ascorbic acid. 2% acetic acid, 1% lactic acid and 0.25% citric acid) to reduce bacterial counts. and precured with 10% NaCl, then the traditional methods was followed.

Lactic acid bacteria inoculation: Precured pork was mixed with a culture of 3×10^6 cfu/g of lactic acid bacteria (*Lactobacillus plantarum* CCRC 12327, FIRDI. Taiwan). Then the traditional technique was followed and the product was sealed for fermentation of lactic acid bacteria.

Analytical procedures

Water activity (Aw) of the products was determined with a Rotronic Hydroskop DST (Switzland) and pH values were measured directly using a pH-meter (model 6200. Jenco. Taiwan) with an insertion-type electroprobe. Sodium chloride content was determined with the Barnstein method and protein by the Kjeldahl method (AOAC. 1984).

The aerobic bacterial counts, anaerobic bacterial counts and lactic acid bacterial counts were determined by the methods of FDA (FDA. 1976). *Clostridium botulinum* was measured by the method of Imai et al. (1990). Trichinella spiralis was detected according to the method of Childers et al. (1982).

Amines were determined by the methods of Yen (1986). Nucleotides were analyzed by the method of Boyle et al. (1991) and Ryder (1985). Five grams of meat sample were extracted with 25 ml of 0.6 M $HClO_4$. The $HClO_4$ extract was adjusted to pH 6.5-6.8 with 1M KOH and filtered through a membrane filter (pore size 0.45 μm). The procedure of high performance liquid chromatography (HPLC) analysis of ATP and its related compounds were modified according to the methods of Boyle et al. (1991) and Ryder (1985). The HPLC chromatography was performed on a Model L6200 (Hitachi Co.. Japan). Intelligent pump equipped with a model L-4200 UV-V lamp was used as a detector for monitoring at 260 μm (Hitachi Co.. Japan).

Sensory characteristics of Si-raw appearance, taste. saltiness, tenderness and overall acceptance were evaluated by using a Hedonic system (7 points scale: like=7. dislike=1). The panelists were untrained and the test was performed by three different groups of people (Ten students working in the lab. and 30 students of Taiwan Provincial Jenai Agricultural Professional High School and 12 aborigines from Jenai, Nantou country). The data were analyzed using a SAS statistical program. Means separation was conducted by using the technique of Duncan (SAS. 1988).

RESULTS AND DISCUSSION

The result presented in Table 1 revealed that all treatments could decrease water activity (Aw) of Si-Raw from 0.97-0.98 of fresh meat to approximately 0.92. However. there were also differences among the treatments. It was found that the Aw for the traditional was not different from the treatment with 3% Na-hypophosphite. but significantly different from other treatments ($p>0.05$). The difference in Aw between the treatments with plum paste and anka mash was also not significant. but they were significantly different from other treatments ($p>0.05$). The salt concentration of Si-Raw samples from the traditional. citric acid. sodium hypophosphite plus citric acid. *Monascus anka* mash, plum paste, organic acid sprayed and lactic acid bacteria inoculation methods were 6.67%. 6.02%. 6.20%. 6.29%. 6.36%. 5.46%. 6.49%. respectively. It is recognized that the effect of low Aw (below 0.93-0.94) inhibits *Cl. botulinum* growth (Baird-Parker and Freame. 1967; Haushild. 1989). Thus. the data presented in Table 1 suggest that the level of Aw for all treatments might inhibit

Table 1. Water activity, water activity (Aw), pH and salt content of Si-Raw*

Treatments	Water activity (Aw)	Salt (%)	pH
Traditional	$0.922\pm0.0005^{a*}$	6.7 ± 0.07^{a}	4.27 ± 0.03^{a}
Citric acid	0.939 ± 0.0038^{d}	6.0 ± 0.06^{b}	4.18 ± 0.07^{cd}
Na-hypophosphite	0.922 ± 0.0020^{a}	6.2 ± 0.04^{c}	4.13 ± 0.03^{def}
Monascus anka mash	0.913 ± 0.0023^{c}	6.3 ± 0.07^{d}	4.20 ± 0.04^{bc}
Plum paste	0.915 ± 0.0018^{e}	6.4 ± 0.07^{e}	4.24 ± 0.02^{ab}
Sprayed with organic acid	0.932 ± 0.0021^{d}	5.5 ± 0.04^{f}	4.12 ± 0.04^{ef}
Lactic acid bacteria	0.926 ± 0.0016^{e}	6.5 ± 0.09^{g}	4.08 ± 0.01^{f}

* Figures in the same column with the same letters are not significantly different. ($p>0.05$).

Cl. botulinum growth. Raw meat precured with 10% salt could decrease the Aw level to approximately 0.95, and the Aw for the different treatments under anaerobic fermentation decreased to below 0.94. This indicated that all treatments were apparently effective in decreasing the Aw of the Si-Raw product.

The pH value (4.08) for the samples inoculated with *Lactobacillus plantarum* was the lowest among all treatments. Table 1 also shows that the pH of all treatments dropped from 6.7 to 4.3 which might inhibit *Cl. botulinum* growth. There were significant differences ($p>0.01$) between the traditional sample and other treatments except the product in which plum paste was added. As mentioned of Schillinger and Lucke (1990) had been isolated 221 species of lactic acid bacteria from meat and meat products. There are some lactic acid bacteria naturally growing on meat surfaces. The difference in pH values among the treatments might be due to naturally occurring lactic acid bacteria causing a different rate of acid production. In Taiwan, Si-Raw is cured in a container and sealed leading to anaerobic fermentation. Thus, the pH value lowering of the products is caused by microbial action. Otherwise, steamed rice is added to cured meat to supply a carbon source as Si-Raw is manufactured.

It is generally recognized that the factors affecting growth and toxin production of *Cl. botulinum* are pH, composition of the food, reduction oxidation (redox) potential and presence of inhibitors (such as nitrite and nisin), Aw, temperature and competitive flora (Glass and Doyle, 1991; Haushild and Hilshimer, 1979). As the pH drops below 4.6 and the redox potential increases above 150 mV, *Cl. botulinum* growth is inhibited. On the other hand, as Aw is lowered below 0.93-0.92 the organisms are incapable of growth (Baird-Parker and Freame, 1967; Haushild, 1989). Over all Si-Raw is generally recognized as safe because it has at least three factors inhibiting *Cl. botulinum* growth, the factors including low Aw value, reduced pH and inhibitory agents, such as food additives. The control of sanitation conditions is also one of the major concerns in the process of food production. The effectiveness of the various treatments to inhibit toxin generation in cases of *Cl. botulinum* contamination in the production will need to be investigated by further inoculation tests.

The concentrations of nonprotein nitrogen (NPN), amino acid nitrogen (AAN) and amines are shown in Table 2, indicating the changes in muscle proteins during fermentation. Proteolytic enzymes break down muscle proteins producing NPN compounds that contribute to flavor and slightly increase the pH (Demeyer et al., 1979; Mihalyi and Kormendy, 1967). The result indicated that the NPN content of sample from raw meat sprayed with organic acids was the highest, and the anka mash treated sample was second and the traditional sample was third in order.

Table 2. Nonprotein nitrogen (NPN) and amino acid nitrogen (AAN) contents of Si-Raw*

	NPN (%)	AAN (%)
Traditional	0.83	0.18 ± 0.07^{ac}
Citric acid	0.63	0.16 ± 0.02^{ac}
Na-hypophosphite	0.61	0.16 ± 0.02^{ac}
Monascus anka mash	0.89	0.54 ± 0.02^{b}
Plum paste	0.67	0.13 ± 0.03^{c}
Sprayed with organic acid	1.26	0.17 ± 0.03^{c}
Lactic acid bacteria	0.71	0.21 ± 0.09^{a}

* Figures in the same column with the same letters are not significantly different. ($p>0.05$).

Amino acid nitrogen content was highest in the sample treated with anka mash, and the traditional sample was second, lactic acid bacteria inoculated sample was third in order. The data also indicated that the NPN and AAN contents of anka mash treated samples were higher than other treatments. These findings might be caused by initial concentrations of NPN and amino nitrogen in the anka mash. *Monascus anka* has proteolytic activity as reported by Tseng (1987) and Lin (1986).

As a result of NPN and AAN contents, it could be noted that NPN content of the sample inoculated with lactic acid bacteria had the same trend as the traditional process and the sample from raw meat sprayed with organic acid was higher, but AAN was not significantly lower than traditional method. These results indicated that the quality of this sample was undesirable. There was a very significant difference in amino acid nitrogen contents between the anka mash added sample and the other treatments ($p>0.05$), but the rest of the treatments except anka mash added sample were not significantly different ($p>0.05$).

It could be noted that the treatments caused some differences in the levels and kinds of amines resulting from the fermentation in Si-Raw products (Table 3). Food scientists pay more attention to the levels of biogenic amines present in food since it has been demonstrated that these compounds are harmful. Biogenic amines are either psychoactive or vasoactive and may cause problems with some consumers (Lovenberg, 1974). In general, decarboxylation of amino acids is caused by microbial decarboxylase which produces biogenic amines (Yen, 1986; Yen and Weo, 1990). Although these amines such as putrescine, cadaverine, histamine, bata-phenylethamine, spermine, spermidine, tryptamine and tyramine have been reported higher in some fermented foods. In this study, except for tryptamine concentration in the samples containing *Monascus anka* mash, amine concentrations of all the treatments were lower when compared with those of other fermented meat products (Yamanaka, 1989; Lin, 1986; Lakritz et al., 1975). Although AAN content was higher in the product (Table 2) produced from raw meat treated with organic acids, this value was lower in products

Table 3. Concentration (*n*g/g) of biogenic amines in Si-Raw

	Putrescine	Cadaverine	Trypatamine	β-phenylethamine	Spermidine	Spermine	Histamine	Tyramine	Agmatine
Traditional	ND	ND	ND	0.9-27.1[A] 21.8[B]	ND-3.3 0.8	1.7-43.9 13.2	1.0-34.0 7.6	9.2-375.8 94.9	ND-313.7 110.5
Citric acid	ND	ND	ND-7.0 5.0	ND-103.6 41.7	ND-186.0 100.3	12.6-20.9 17.1	ND-3.8 1.3	ND-137.4 29.9	ND
Na-hypophosphite	ND	ND-1.3 0.0	ND-5.5 2.3	ND-51.2 8.7	ND-166.0 66.1	2.4-56.1 30.8	ND-105.2 36.3	ND-189.8 43.7	ND-220 59.9
Monascus anka mash	ND-2.4 0.48	ND	0.4-92.8 62.2	0.1-74.1 15.6	ND-5.5 2.0	4.61-56.9 17.6	2.4-21.4 15.4	21.5-271.3 87.71	ND-3.2 1.0
Plum paste	ND	ND-5.8 1.2	ND	ND-488.8 83.7	ND-0.5 0.1	2.0-93.9 24.0	ND-86.7 25.1	26.3-220.3 75.1	50.6-299.2 42.2
Sprayed with organic acid	ND-11.3 3.25	ND-11.6 1.9	ND-14.4 5.0	ND-0.1 0.0	ND-28.0 0.3	ND-23.3 10.6	ND-26.15 3.86	4.6-257.4 79.7	ND-127 21.8
Lactic acid bacteria	ND-1.5 0.3	ND-1.9 0.9	ND-1.9 0.9	ND-0.5 0.0	ND-320.8 65.3	ND-1.5 2.0	ND-4.1 0.9	3.8-170.5 52.6	ND-44.2 6.3

ND: Not detected. A: range, B: mean.

obtained from other treatments.

Table 4 indicates the results of the effect of treatments on ATP and its related compounds. ATP, AMP and hypoxanthine levels from the analysis of variance indicated a very significant differences among the treatments ($p>0.01$). ATP concentration was higher in the samples which contained anka mash and those with lactic acid bacteria. This might explain the ATP level which originated from the sample treated with microbial cells. AMP in the anka mash treated sample was significantly higher ($p>0.05$) than that of the other treatments. Hypoxanthine in the sample of the traditional, organic acid sprayed, Na-hypophosphite and citric acid were not significantly different ($p>0.05$), while the other treatments were significantly different ($p>0.05$). Hypoxanthine of the sample with citric acid was lowest and its inosine content was the highest. This aspect might be caused by the initial pH in the early period of fermentation which may have inactivated the enzymatic activity resulting in inosine levels of the sample with citric acid being higher than that of the other treatments (Sung et al., 1976). Depletion of ATP in muscle is due to postmortem time, but the results are not in agreement with the changes expected. In general, IMP content was at lower levels and ATP and hypoxanthine remained at higher levels. It was known that the later stage of fermentation would favor lactic acid bacteria growth under anaerobic condition and increase the microbial ATP (Prescott et al., 1990; Moat and Foster, 1988). Our results suggested that the ATP content of Si-Raw was increased during the final stage of fermentation. The higher level of ATP might be the result of the decrease in ATP degradation resulted from the inhibition of ATPase in higher salt concentration and low pH value in the product (Sung et al., 1979). Additionally, ATP production by substrate-level phosphorylation of lactic acid bacteria (Prescott et al., 1990; Moat and Foster, 1988) with tolerance to high salt conditions might also contribute to the high ATP content in the Si-Raw product.

Neither *Trichinella spiralis* nor *Cl. botulinum* was detected in any treatments. The pH value, Aw, and salt content of the final Si-Raw products might control *Trichinella spiralis*. The results are in agreement with the report from Crouse and Kemp (1969) who indicated that *Trichinella spiralis* was not detected when pork was cured with 6% salt, and smoked, then aged at 25°C for two weeks. Lotzch and Rodel (1974) reported that if the Aw fell below 0.949 *Trichinella spiralis* could not survive in sausage, but they suggested that it was better to be maintained the level below 0.94. Figure 1 showed that anaerobic bacterial counts, lactic acid bacterial counts and aerobic bacterial counts for

Table 4. Concentration (mg/kg) of ATP and its related compounds in Si-Raw

	ATP	ADP	AMP	IMP	Inosine	Hypoxanine
Traditional	1.107±0.003^{d*}	0.015±0.005^{b}	ND	ND	ND	0.279±0.003^{b}
Citric acid	1.123±0.010^{cd}	ND	0.016±0.004^{b}	ND	0.420±0.034^{a}	0.144±0.003^{d}
Na-hypophosphite	1.150±0.030^{cd}	ND	0.012±0.006^{b}	ND	ND	0.271±0.007^{b}
Monascus anka mash	1.261±0.039^{a}	0.004±0.001^{c}	0.152±0.004^{a}	0.026±0.003	0.007±0.040^{b}	0.341±0.011^{a}
Plum paste	1.021±0.072^{e}	0.043±0.002^{a}	ND	ND	0.077±0.011^{b}	0.233±0.013^{c}
Sprayed with organic acid	1.185±0.050^{bc}	ND	0.014±0.004^{b}	ND	ND	0.272±0.013^{b}
Lactic acid bacteria	1.221±0.035^{ab}	ND	0.013±0.009^{b}	ND	ND	0.267±0.005^{b}

* Figures in the same column with the same letters are not significantly different ($p>0.05$).
Initial ATP contents<0.1 mg/kg. Initial NaCl contents<0.29%.
ND: Not detected.

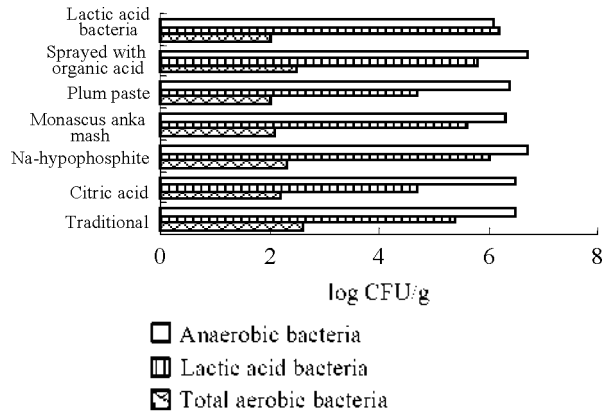

Figure 1. Total aerobic bacterial count, lactic acid bacterial count and anaerobic bacterial count in Si-Raw (raw cured fermented meat).

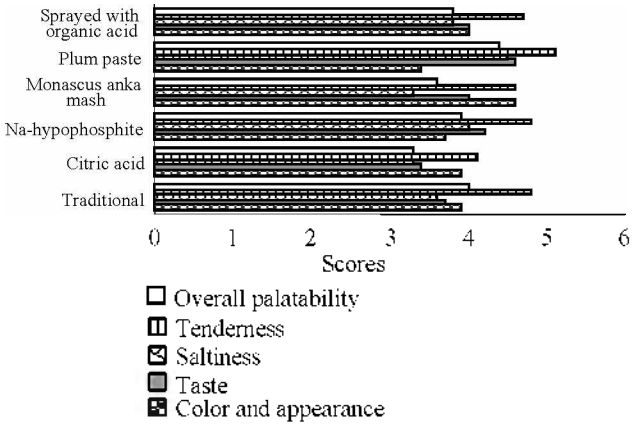

Figure 2. Scores of sensory characteristics of Si-Raw evaluated by the students working in meat Lab. Scores based on 7-point scale (7=like, 1=dislike).

the products of all treatments were less than 10^6, 10^5 and 10^2 cfu/g, respectively. Total aerobic bacteria and anaerobic bacteria were the dominant flora in the raw cured fermented meat. This result might be due to the mesophilic aerobic bacteria growth being inhibited by the environmental factors such as redox potential, reduced pH, high salt content, reduced Aw, and competitors. To our knowledge, there are no related data concerning this topic, since this type of research has not been reported previously. Therefore, hedonic system (7 points scale) was utilized in this study to compare the preferences of panelists. Three different groups the aborigines, professional high school students and the students working in the research laboratory conducted the sensory evaluation. All products were acceptable to the aborigines and agricultural school students but some were not acceptable to the research students. However, the samples with Na-hypophosphite were the most acceptable to most of the test panelists (See Figures 2, 3 and 4). The results also indicated that the Taiwan aborigines had a higher preference to Si-Raw, probably because that they have become accustomed to the special flavor of Si-Raw. In contrast, the college students with knowledge background of meat production showed less favorable acceptance, since they are not adapted to uncooked cured meat and the sour taste of the product.

Fermented meat products are generally recognized as safe. As pointed out earlier these factors such as pH, Aw value play a major role, although other factors could also contribute to the safety of the raw fermented meat products (Mandigo, 1992; Incze, 1992; Smith and Palumbo, 1981). Reports from several researches also indicated that hurdle technology, pH value, Aw, saltiness and competitors are the inhibitory factors or hurdle for microbial growth and subsequently obtaining safety (Hecheiumann and Kasprowiak, 1991; Leistner and Rodel, 1976). The results of this study seemed to improve the processing method for

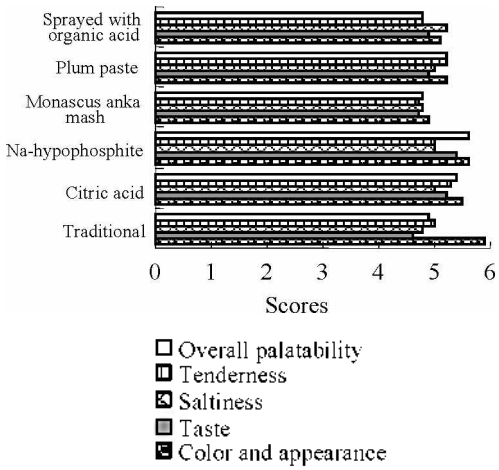

Figure 3. Scores of sensory characteristics of Si-Raw evaluated by the agricultural high school students. Scores based on the same time of evaluated and the same scale as Figure 2.

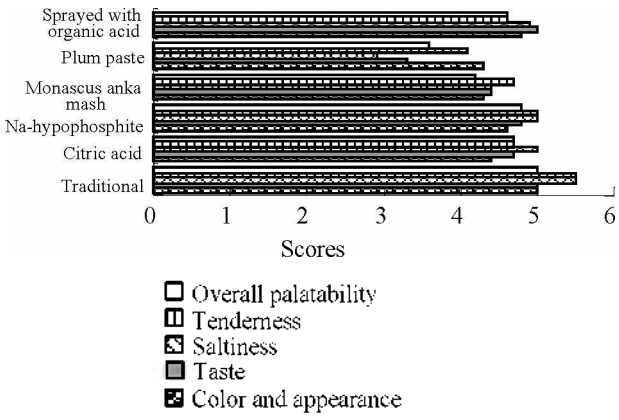

Figure 4. Scores of sensory characteristics of Si-Raw evaluated by the aborigines. Scores based on the same time of evaluated and the same scale as Figure 2.

Si-Raw and enhanced its safety against botulism. As mentioned previously there was no great difference between the traditional method and new methods used in this experiment. Both the traditional method and the new methods could inhibit *Cl. botulinum* growth. However in practice, history would indicate that the traditional methods has a great deal of variability and when the product is poorly produced botulism can be a concern. If critical points are controlled properly, this type of fermented meat products (Si-Raw) should be safe because either low Aw-value or the combination of reduced Aw-value and reduced pH inhibited the growth of undesirable microorganisms.

ACKNOWLEDGMENTS

The authors are grateful to Department of Health, Executive Yuan of Republic of China for financial support.

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